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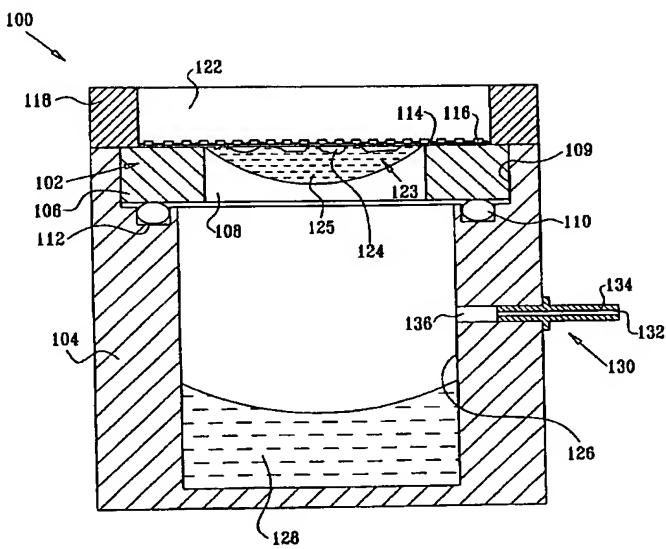
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(54) Title: LOW-PRESSURE CHAMBER FOR SCANNING ELECTRON MICROSCOPY IN A WET ENVIRONMENT



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(57) Abstract: A specimen enclosure assembly for use in an electron microscope and including a rigid specimen enclosure dish having an aperture and defining an enclosed specimen placement volume, an electron beam permeable, fluid impermeable, cover sealing the specimen placement volume at the aperture from a volume outside the enclosure and a pressure controller communicating with the enclosed specimen placement volume and being operative to maintain the enclosed specimen placement volume at a pressure which exceeds a vapor pressure of a liquid sample in the specimen placement volume and is greater than a pressure of a volume outside the enclosure, whereby a pressure differential across the cover does not exceed a threshold level at which rupture of the cover would occur. 2

## LOW-PRESSURE CHAMBER FOR SCANNING ELECTRON MICROSCOPY IN A WET ENVIRONMENT

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### REFERENCE TO CO-PENDING APPLICATIONS

Applicant hereby claims priority of Israel Patent Application Serial No. 150056, filed on June 5, 2002, entitled "Low Pressure Chamber for Scanning Electron Microscopy in a Wet Environment".

### FIELD OF THE INVENTION

The present invention relates to specimen enclosures for scanning electron microscope (SEM) inspection systems and more particularly to fluid specimen enclosures.

### BACKGROUND OF THE INVENTION

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The following patent documents are believed to represent the current state of the art:

4,071,766; 4,720,633; 5,250,808; 5,326,971; 5,362,964; 5,412,211;  
4,705,949; 5,945,672; 6,365,898; 6,130,434; 6,025,592; 5,103,102; 4,596,928;  
25 4,880,976; 4,992,662; 4,720,622; 5,406,087; 3,218,459; 3,378,684; 4,037,109;  
4,448,311; 4,115,689; 4,587,666; 5,323,441; 5,811,803; 6,452,177; 5,898,261;  
4,618,938; 6,072,178; 4,929,041 and 6,114,695.

## SUMMARY OF THE INVENTION

The present invention seeks to provide apparatus and systems for enabling scanning electron microscope inspection of fluid containing specimens.

5 There is thus provided in accordance with a preferred embodiment of the present invention a specimen enclosure assembly for use in an electron microscope and including a rigid specimen enclosure dish having an aperture and defining an enclosed specimen placement volume, an electron beam permeable, fluid impermeable, cover sealing the specimen placement volume at the aperture from a volume outside the  
10 enclosure and a pressure controller communicating with the enclosed specimen placement volume and being operative to maintain the enclosed specimen placement volume at a pressure which exceeds a vapor pressure of a liquid sample in the specimen placement volume and is greater than a pressure of a volume outside the enclosure, whereby a pressure differential across the cover does not exceed a threshold level at  
15 which rupture of the cover would occur.

In accordance with another preferred embodiment of the present invention the pressure controller includes a passageway communicating with the enclosed specimen placement volume. Preferably, the passageway includes a tube having a lumen whose cross section is sufficiently small as to maintain the pressure, which exceeds the vapor pressure of the liquid sample in the specimen placement volume and is greater than the pressure of the volume outside the enclosure, for a time period of at least 15 minutes. Additionally, the tube communicates with a fluid reservoir.

25 In accordance with yet another preferred embodiment of the present invention the specimen enclosure assembly also includes a liquid ingress and egress assembly permitting supply and removal of liquid from the enclosed specimen placement volume. Preferably, the liquid ingress and egress assembly includes at least two tubes.

30 There is also provided in accordance with another preferred embodiment of the present invention a specimen enclosure assembly for use in an electron microscope and including a rigid specimen enclosure dish having an aperture and defining an enclosed specimen placement volume, an electron beam permeable, fluid

impermeable, cover sealing the specimen placement volume at the aperture from a volume outside the enclosure and a liquid ingress and egress assembly permitting supply and removal of liquid from the enclosed specimen placement volume.

5 Preferably, the liquid ingress and egress assembly includes at least two tubes.

There is further provided in accordance with yet another preferred embodiment of the present invention a scanning electron microscope assembly including a scanning electron microscope defining an examination volume, a specimen enclosure assembly disposed in the examination volume and including a rigid specimen 10 enclosure dish having an aperture and defining an enclosed specimen placement volume, an electron beam permeable, fluid impermeable, cover sealing the specimen placement volume at the aperture from a volume outside the enclosure and a pressure controller communicating with the enclosed specimen placement volume and being operative to maintain the enclosed specimen placement volume at a pressure which 15 exceeds a vapor pressure of a liquid sample in the specimen placement volume and is greater than a pressure of a volume outside the enclosure, whereby a pressure differential across the cover does not exceed a threshold level at which rupture of the cover would occur.

20 In accordance with another preferred embodiment of the present invention the pressure controller includes a passageway communicating with the enclosed specimen placement volume. Preferably, the passageway includes a tube having a lumen whose cross section is sufficiently small as to maintain the pressure, which exceeds the vapor pressure of the liquid sample in the specimen placement 25 volume and is greater than the pressure of the volume outside the enclosure, for a time period of at least 15 minutes. Additionally, the tube communicates with a fluid reservoir.

30 In accordance with yet another preferred embodiment of the present invention the scanning electron microscope also includes a liquid ingress and egress assembly permitting supply and removal of liquid from the enclosed specimen placement volume. Preferably, the liquid ingress and egress assembly includes at least two tubes.

There is also provided in accordance with yet another preferred

embodiment of the present invention a specimen enclosure assembly for use in an electron microscope and including a fluid reservoir, a plurality of rigid specimen enclosure dishes, each having an aperture and defining an enclosed specimen placement volume, the plurality of rigid specimen enclosure dishes communicating with the fluid reservoir, an electron beam permeable, fluid impermeable, cover sealing each of the specimen placement volumes at the apertures from a volume outside each of the enclosures, and a pressure controller communicating with the fluid reservoir and being operative to maintain the enclosed specimen placement volumes at a pressure which exceeds a vapor pressure of a liquid sample in the specimen placement volumes and is greater than a pressure of a volume outside the fluid reservoir, whereby a pressure differential across the covers does not exceed a threshold level at which rupture of the covers would occur.

In accordance with another preferred embodiment of the present invention the pressure controller includes a passageway. Preferably, the passageway includes a tube having a lumen whose cross section is sufficiently small as to maintain the pressure, which exceeds the vapor pressure of the liquid sample in the specimen placement volume and is greater than the pressure of the volume outside the plurality of enclosures, for a time period of at least 15 minutes. Additionally, the tube communicates with the fluid reservoir. Additionally or alternatively, the specimen enclosure assembly also includes a liquid ingress and egress assembly permitting supply and removal of liquid from the enclosed specimen placement volume. Preferably, the liquid ingress and egress assembly includes at least two tubes.

## BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be understood and appreciated more fully  
5 from the following detailed description, taken in conjunction with the drawings in  
which:

Fig. 1 is a simplified sectional illustration of a specimen enclosure  
assembly constructed and operative in accordance with a preferred embodiment of the  
present invention;

10 Fig. 2 is a simplified sectional illustration of a specimen enclosure  
assembly constructed and operative in accordance with another preferred embodiment  
of the present invention;

15 Fig. 3 is a simplified sectional illustration of a specimen enclosure  
assembly constructed and operative in accordance with yet another preferred  
embodiment of the present invention;

Fig. 4 is a simplified sectional illustration of a multiple specimen  
enclosure assembly constructed and operative in accordance with a preferred  
embodiment of the present invention;

20 Fig. 5 is a simplified pictorial and sectional illustration of a scanning  
electron microscope including the specimen enclosure assembly of Fig. 1;

Fig. 6 is a simplified pictorial and sectional illustration of a scanning  
electron microscope including the specimen enclosure assembly of Fig. 2;

Fig. 7 is a simplified pictorial and sectional illustration of a scanning  
electron microscope including the specimen enclosure assembly of Fig. 3; and

25 Fig. 8 is a simplified pictorial and sectional illustration of a scanning  
electron microscope including the multiple specimen enclosure assembly of Fig. 4.

## DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Reference is now made to Fig. 1, which is a simplified sectional illustration of a specimen enclosure assembly 100 constructed and operative in accordance with a preferred embodiment of the present invention. As seen in Fig. 1, the specimen enclosure assembly 100 comprises a specimen enclosure dish 102 seated in a container 104.

Specimen enclosure dish 102 preferably is formed of a ring 106 having a generally central aperture 108. Ring 106 is preferably formed of PMMA (polymethyl methacrylate), such as Catalog No. 692106001000, commercially available from Irpen S.A. of Barcelona, Spain, and preferably defines a specimen placement enclosure with a volume of approximately 20 microliters and a height of approximately 2 mm. The specimen enclosure dish 102 is seated in a recess 109 formed in a top of the container 104.

An O-ring 110 is preferably disposed between ring 106 and an interior surface 112 of container 104.

An electron beam permeable, fluid impermeable, cover 114 is placed on specimen enclosure dish 102 against and over central aperture 108.

The electron beam permeable, fluid impermeable, cover 114 preferably comprises a polyimide membrane, such as Catalog No. LWN00020, commercially available from Moxtek Inc. of Orem, UT, U.S.A. Cover 114 is adhered, as by an adhesive, to a mechanically supporting grid 116, which is not shown to scale, such as Catalog No. 2007N or Catalog No. 2005N, which is commercially available from Structure Probe Inc. of 569 East Gay Street, West Chester, PA, U.S.A. A preferred adhesive is commercially available from Norland Products Inc. of Cranbury, NJ, U.S.A., identified by Catalog No. NOA61. The electron beam permeable, fluid impermeable, cover 114 is also adhered to ring 106, preferably by an adhesive, such as Catalog No. NOA61, commercially available from Norland Products Inc. of Cranbury, NJ, U.S.A.

A top element 118 is provided to retain the specimen enclosure dish 102 in container 104. Top element 118 is preferably formed as a ring having a generally

central aperture 122 and is attached to container 104 by any conventional means, such as by screws (not shown).

A specimen 123, typically containing cells 124 in a liquid medium 125, is typically located within the specimen enclosure dish 102, lying against the electron beam permeable, fluid impermeable, cover 114. Examples of specimens containing liquid may include cell cultures, blood and bacteria. It is noted that the liquid 125 in specimen 123 does not flow out of the specimen enclosure dish 102 due to surface tension.

Container 104 defines a fluid reservoir 126 containing at least one fluid. The fluid preferably comprises a liquid 128, such as water or specimen liquid. The liquid 128 in fluid reservoir 126 is provided to supply the specimen enclosure dish 102 with vapor, such as water vapor, so as to prevent evaporation of the specimen liquid 125 by permitting vapor flow into specimen enclosure dish 102 through aperture 108.

A pressure controller assembly 130 is operative to maintain the specimen enclosure dish 102, during microscopic inspection, generally over a time duration in a range of several minutes to several hours, typically a time period of at least 15 minutes, at a pressure which exceeds a vapor pressure of the specimen 123 and is greater than a pressure of a volume outside the specimen enclosure assembly 100, whereby a pressure differential across the electron beam permeable, fluid impermeable, cover 114 does not exceed a threshold level at which rupture of cover 114 would occur.

The pressure controller assembly 130 preferably comprises a tube 132, such as Catalog No. MF34G-5 or Catalog No. MF28G-5, commercially available from World Precision Instruments Inc. of 175 Sarasota Center Boulevard, Sarasota, Florida, U.S.A., and a tube housing 134.

Tube 132 is inserted into an aperture 136 formed in a wall of container 104 above a surface of the liquid 128 in the fluid reservoir 126. Tube 132 is sealingly attached to the container wall so that fluid flows from container 104 only through the tube 132. It is a particular feature of the present invention that the tube 132 has a lumen with a cross section sufficiently small, preferably of a diameter in a range of 50 to 150 micrometers, to provide for relatively slow dissipation of pressure from the specimen enclosure assembly 100.

Reference is now made to Fig. 2, which is a simplified sectional

illustration of a specimen enclosure assembly 200 constructed and operative in accordance with another preferred embodiment of the present invention. As seen in Fig. 2, the specimen enclosure assembly 200 comprises a specimen enclosure dish 202 seated in a container 204.

5 Specimen enclosure dish 202 preferably is formed of a ring 206 having a generally central aperture 208. Ring 206 is preferably formed of PMMA (polymethyl methacrylate), such as Catalog No. 692106001000, commercially available from Irpen S.A. of Barcelona, Spain, and preferably defines a specimen placement enclosure with a volume of approximately 20 microliters and a height of approximately 2 mm. The 10 specimen enclosure dish 202 is seated in a recess 209 formed in a top of the container 204.

An O-ring 210 is preferably disposed between ring 206 and an interior surface 212 of container 204.

15 An electron beam permeable, fluid impermeable, cover 214 is placed on specimen enclosure dish 202 against and over central aperture 208.

The electron beam permeable, fluid impermeable, cover 214 preferably comprises a polyimide membrane, such as Catalog No. LWN00020, commercially available from Moxtek Inc. of Orem, UT, U.S.A. Cover 214 is adhered, as by an adhesive, to a mechanically supporting grid 216, which is not shown to scale, such as 20 Catalog No. 2007N or Catalog No. 2005N, which is commercially available from Structure Probe Inc. of 569 East Gay Street, West Chester, PA, U.S.A. A preferred adhesive is commercially available from Norland Products Inc. of Cranbury, NJ, U.S.A., identified by Catalog No. NOA61. The electron beam permeable, fluid impermeable, cover 214 is also adhered to ring 206, preferably by an adhesive, such as 25 Catalog No. NOA61, commercially available from Norland Products Inc. of Cranbury, NJ, U.S.A. A top element 218 is provided to retain the specimen enclosure dish 202 in container 204. Top element 218 is preferably formed of as a ring having a generally central aperture 222 and is attached to container 204 by any conventional means, such as by screws (not shown).

30 A specimen 223, typically containing cells 224 in a liquid medium 225, is typically located within the specimen enclosure dish 202, lying against the electron

beam permeable, fluid impermeable, cover 214. Examples of specimens containing liquid may include cell cultures, blood and bacteria.

Container 204 contains at least one fluid. The fluid preferably comprises a liquid 228, such as water or specimen liquid. Liquid 228 typically fills container 204 and specimen enclosure dish 202, as shown in Fig. 2, or fills part of container 204, similar to reservoir 126 shown in Fig. 1. The liquid 228 in container 204 is provided to supply the specimen enclosure dish 202 with vapor, such as water vapor, so as to prevent evaporation of the specimen liquid 225.

A fluid reservoir 230 contains at least one fluid in addition to the fluid contained in the specimen enclosure assembly 200. The fluid preferably comprises a liquid 232, such as water or specimen liquid. Preferably, fluid reservoir 230 has a larger internal volume than specimen enclosure assembly 200. A lid 234 covers fluid reservoir 230 and is attached to fluid reservoir 230 by any conventional means, such as by screws (not shown). The liquid 232 in fluid reservoir 230 is provided to supply the specimen enclosure assembly 200 with additional vapor, such as water vapor, in addition to the container liquid 228, so as to further prevent evaporation of the specimen liquid 225, by permitting vapor flow into specimen enclosure dish 202 through a fluid passageway 240.

The fluid passageway 240 comprises a conduit 242 having a first end portion and a second end portion, designated by reference numerals 244 and 246 respectively. First end portion 244 is inserted into an aperture 248 formed in a wall of container 204 and second end portion 246 is inserted into an aperture 250 formed in a wall of fluid reservoir 230.

A pressure controller assembly 260 is operative to maintain the specimen enclosure dish 202, during microscopic inspection, generally over a time duration in a range of several minutes to several hours, typically a time period of at least 15 minutes, at a pressure which exceeds a vapor pressure of the specimen 223 and is greater than a pressure of a volume outside the specimen enclosure assembly 200, whereby a pressure differential across the electron beam permeable, fluid impermeable, cover 214 does not exceed a threshold level at which rupture of cover 214 would occur. Additionally, the fluid in fluid reservoir 230 is provided to further maintain the pressure within the

specimen enclosure assembly 200, as described hereinabove, during microscopic inspection.

The pressure controller assembly 260 preferably comprises a tube 262, such as Catalog No. MF34G-5 or Catalog No. MF28G-5, commercially available from 5 World Precision Instruments Inc. of 175 Sarasota Center Boulevard, Sarasota, Florida, U.S.A., and a tube housing 264.

Tube 262 is inserted into an aperture 266 formed in the fluid reservoir wall above a surface of the liquid 232 in the fluid reservoir 230. Tube 262 is sealingly attached to the fluid reservoir wall so that fluid flows from fluid reservoir 230 only 10 through the tube 262 and fluid passageway 240. It is a particular feature of the present invention that the tube 262 has a lumen with a cross section sufficiently small, preferably of a diameter in a range of 50 to 150 micrometers, to provide for relatively slow dissipation of pressure from the fluid reservoir 230.

Reference is now made to Fig. 3, which is a simplified sectional illustration of a specimen enclosure assembly 300 constructed and operative in accordance with yet another preferred embodiment of the present invention. As seen in 15 Fig. 3, the specimen enclosure assembly 300 comprises a specimen enclosure dish 302 seated in a container 304.

Specimen enclosure dish 302 preferably is formed of a ring 306 having a 20 generally central aperture 308. Ring 306 is preferably formed of PMMA (polymethyl methacrylate), such as Catalog No. 692106001000, commercially available from Irpen S.A. of Barcelona, Spain, and preferably defines a specimen placement enclosure with a volume of approximately 20 microliters and a height of approximately 2 mm. The specimen enclosure dish 302 is seated in a recess 309 formed in a top of the container 25 304.

An O-ring 310 is preferably disposed between ring 306 and an interior surface 312 of container 304.

An electron beam permeable, fluid impermeable, cover 314 is placed on specimen enclosure dish 302 against and over central aperture 308.

The electron beam permeable, fluid impermeable, cover 314 preferably 30 comprises a polyimide membrane, such as Catalog No. LWN00020, commercially available from Moxtek Inc. of Orem, UT, U.S.A. Cover 314 is adhered, as by an

adhesive, to a mechanically supporting grid 316, which is not shown to scale, such as Catalog No. 2007N or Catalog No. 2005N, which is commercially available from Structure Probe Inc. of 569 East Gay Street, West Chester, PA, U.S.A. A preferred adhesive is commercially available from Norland Products Inc. of Cranbury, NJ, U.S.A., identified by Catalog No. NOA61. The electron beam permeable, fluid impermeable, cover 314 is also adhered to ring 306, preferably by an adhesive, such as Catalog No. NOA61, commercially available from Norland Products Inc. of Cranbury, NJ, U.S.A. A top element 318 is provided to retain the specimen enclosure dish 302 in container 304. Top element 318 is preferably formed as a ring having a generally central aperture 322 and is attached to container 304 by any conventional means, such as by screws (not shown).

A specimen 323, typically containing cells 324 in a liquid medium 325, is typically located within the specimen enclosure dish 302, lying against the electron beam permeable, fluid impermeable, cover 314. Examples of specimens containing liquid may include cell cultures, blood and bacteria.

Container 304 contains a liquid 328, such as water or specimen liquid, filling container 304 and the specimen enclosure dish 302.

A fluid reservoir 330 contains at least one fluid in addition to the fluid contained in the specimen enclosure assembly 300. The fluid preferably comprises a liquid 332, such as water or specimen liquid. Preferably, fluid reservoir 330 has a larger internal volume than specimen enclosure assembly 300. A lid 334 covers fluid reservoir 330 and is attached to fluid reservoir 330 by any conventional means, such as by screws (not shown). The liquid 332 in fluid reservoir 330 is provided to supply the specimen enclosure assembly 300 with additional vapor, such as water vapor, in addition to the container liquid 328, so as to further prevent evaporation of the specimen liquid 325, by permitting vapor flow into specimen enclosure dish 302 through a fluid passageway 340.

The fluid passageway 340 comprises a conduit 342 having a first end portion and a second end portion, designated by reference numerals 344 and 346 respectively. First end portion 344 is inserted into an aperture 348 formed in a wall of container 304 and second end portion 346 is inserted into an aperture 350 formed in a wall of fluid reservoir 330.

A pressure controller assembly 360 is operative to maintain the specimen enclosure dish 302, during microscopic inspection, generally over a time duration in a range of several minutes to several hours, typically a time period of at least 15 minutes, at a pressure which exceeds a vapor pressure of the specimen 323 and is greater than a 5 pressure of a volume outside the specimen enclosure assembly 300, whereby a pressure differential across the electron beam permeable, fluid impermeable, cover 314 does not exceed a threshold level at which rupture of cover 314 would occur. Additionally, the fluid in fluid reservoir 330 is provided to further maintain the pressure within the specimen enclosure assembly 300, as described hereinabove, during microscopic 10 inspection.

The pressure controller assembly 360 preferably comprises a tube 362, such as Catalog No. MF34G-5 or Catalog No. MF28G-5, commercially available from World Precision Instruments Inc. of 175 Sarasota Center Boulevard, Sarasota, Florida, U.S.A., and a tube housing 364.

15 Tube 362 is inserted into an aperture 366 formed in the fluid reservoir wall above a surface of the liquid 332 in the fluid reservoir 330. Tube 362 is sealingly attached to the fluid reservoir wall so that fluid flows from fluid reservoir 330 only through the tube 362 and fluid passageway 340. It is a particular feature of the present invention that the tube 362 has a lumen with a cross section sufficiently small, 20 preferably with a diameter in a range of 50 to 150 micrometers, to provide for relatively slow dissipation of pressure from the fluid reservoir 330.

Specimen enclosure assembly 300 is preferably provided with a liquid 25 ingress and egress assembly 370 so as to permit supply and removal of liquid from the specimen enclosure assembly 300 to an environment outside a SEM enclosure wall, here designated by reference numeral 372. Liquid ingress and egress assembly 370 preferably comprises an inlet conduit 374 and an outlet conduit 376 attached to specimen enclosure assembly 300.

Reference is now made to Fig. 4, which is a simplified sectional 30 illustration of a multiple specimen enclosure assembly constructed and operative in accordance with a preferred embodiment of the present invention. As seen in Fig. 4, the multiple specimen enclosure assembly is comprised of a plurality of individual specimen enclosure assemblies 400.

Each specimen enclosure assembly 400 comprises a specimen enclosure dish 402 seated in a container 404.

Specimen enclosure dish 402 preferably is formed of a ring 406 having a generally central aperture 408. Ring 406 is preferably formed of PMMA (polymethyl methacrylate), such as Catalog No. 692106001000, commercially available from Irpen S.A. of Barcelona, Spain, and preferably defines a specimen placement enclosure with a volume of approximately 20 microliters and a height of approximately 2 mm. The specimen enclosure dish 402 is seated in a recess 409 formed in a top of the container 404.

An O-ring 410 is preferably disposed between ring 406 and an interior surface 412 of container 404.

An electron beam permeable, fluid impermeable, cover 414 is placed on specimen enclosure dish 402 against and over central aperture 408.

The electron beam permeable, fluid impermeable, cover 414 preferably comprises a polyimide membrane, such as Catalog No. LWN00020, commercially available from Moxtek Inc. of Orem, UT, U.S.A. Cover 414 is adhered, as by an adhesive, to a mechanically supporting grid 416, which is not shown to scale, such as Catalog No. 2007N or Catalog No. 2005N, which is commercially available from Structure Probe Inc. of 569 East Gay Street, West Chester, PA, U.S.A. A preferred adhesive is commercially available from Norland Products Inc. of Cranbury, NJ, U.S.A., identified by Catalog No. NOA61. The electron beam permeable, fluid impermeable, cover 414 is also adhered to ring 406, preferably by an adhesive, such as Catalog No. NOA61, commercially available from Norland Products Inc. of Cranbury, NJ, U.S.A.

A top element 418 is provided to retain the specimen enclosure dish 402 in container 404. Top element 418 is preferably formed as a ring having a generally central aperture 422 and is attached to container 404 by any conventional means, such as by screws (not shown).

A specimen 423, typically containing cells 424 in a liquid medium 425, is typically located within the specimen enclosure dish 402, lying against the electron beam permeable, fluid impermeable, cover 414. Examples of specimens containing liquid may include cell cultures, blood and bacteria. It is noted that the liquid 425 in

specimen 423 does not flow out of the specimen enclosure dish 402 due to surface tension.

The multiple specimen enclosure assembly, as shown in Fig. 4, also comprises a fluid reservoir 430 containing at least one fluid. The fluid preferably comprises a liquid 432, such as water or specimen liquid. The liquid 432 in fluid reservoir 430 is provided to supply each specimen enclosure assembly 400 with vapor, such as water vapor, so as to prevent evaporation of the specimen liquid 425 by permitting vapor flow into individual specimen enclosure assemblies 400 through apertures 434 formed on a bottom surface of containers 404.

A lid 440 defines an array of specimen enclosure support locations 442. Each specimen enclosure support location 442 is preferably defined by a recess 444 arranged to receive specimen enclosure assemblies 400. Specimen enclosure assemblies 400 are sealingly attached to lid 440, by any conventional means, such as by screws (not shown), so as to prevent dissipation of fluid from lid 440.

Lid 440 covers the fluid reservoir 430 and is attached to fluid reservoir 430 by any conventional means, such as by screws (not shown).

A pressure controller assembly 460 is operative to maintain, during microscopic inspection, generally over a time duration in a range of several minutes to several hours, typically a time period of at least 15 minutes, each specimen enclosure dish 402 at a pressure which exceeds a vapor pressure of the liquid specimen 423 and is greater than a pressure of a volume outside the specimen enclosure assembly 400, whereby a pressure differential across the electron beam permeable, fluid impermeable, cover 414 does not exceed a threshold level at which rupture of cover 414 would occur.

The pressure controller assembly 460 preferably comprises a tube 462, such as Catalog No. MF34G-5 or Catalog No. MF28G-5, commercially available from World Precision Instruments Inc. of 175 Sarasota Center Boulevard, Sarasota, Florida, U.S.A., and a tube housing 464.

Tube 462 is inserted into an aperture 466 formed in a wall of fluid reservoir 430 above a surface of the liquid 432. Tube 462 is sealingly attached to the fluid reservoir wall so that fluid flows from fluid reservoir 430 only through the tube 462. It is a particular feature of the present invention that the tube 462 has a lumen with a cross section sufficiently small, preferably with a diameter in a range of 50 to 150

micrometers, to provide for relatively slow dissipation of pressure from the specimen enclosure assembly 400.

Reference is now made to Fig. 5, which is a simplified pictorial and sectional illustration of a SEM including the specimen enclosure assembly of Fig. 1. As seen in Fig. 5, the specimen enclosure assembly, here designated by reference numeral 500, is engaged with a pressure controller assembly, here designated by reference numeral 502. Specimen enclosure assembly 500 and pressure controller assembly 502 are shown positioned on a stage 504 of a SEM 506.

Reference is now made to Fig. 6, which is a simplified pictorial and sectional illustration of a SEM including the specimen enclosure assembly of Fig. 2. As seen in Fig. 6, the specimen enclosure assembly, here designated by reference numeral 600, is engaged with a fluid reservoir, here designated by reference numeral 602, via a fluid passageway 604. A pressure controller assembly, here designated by reference numeral 608, is engaged with fluid reservoir 602. Specimen enclosure assembly 600 and fluid reservoir 602 are shown positioned on a stage 610 of a SEM 612.

Reference is now made to Fig. 7, which is a simplified pictorial and sectional illustration of a SEM including the specimen enclosure assembly of Fig. 3. As seen in Fig. 7, the specimen enclosure assembly, here designated by reference numeral 700, is engaged with a fluid reservoir, here designated by reference numeral 702, via a fluid passageway 704. A pressure controller assembly, here designated by reference numeral 708, is engaged with fluid reservoir 702. Specimen enclosure assembly 700 and fluid reservoir 702 are shown positioned on a stage 710 of a SEM 712. An inlet conduit 720 and an outlet conduit 722 are attached to the specimen enclosure assembly 700.

Reference is now made to Fig. 8, which is a simplified pictorial and sectional illustration of a SEM including the multiple specimen enclosure assembly of Fig. 4. As seen in Fig. 8, the multiple specimen enclosure assembly, here designated by reference numeral 800, is shown positioned on a stage 802 of a SEM 804. A pressure controller assembly, here designated by reference numeral 808, is engaged with the multiple specimen enclosure assembly 800.

It will be appreciated by persons skilled in the art that the present invention is not limited to what has been particularly shown and described hereinabove. Rather the scope of the present invention includes both combinations and

subcombinations of the various features described hereinabove as well as modifications and variations thereof as would occur to a person of skill in the art upon reading the foregoing specification and which are not in the prior art.

## C L A I M S

1. A specimen enclosure assembly for use in an electron microscope and comprising:
  - 5 a rigid specimen enclosure dish having an aperture and defining an enclosed specimen placement volume;
  - an electron beam permeable, fluid impermeable, cover sealing said specimen placement volume at said aperture from a volume outside said enclosure; and
  - 10 a pressure controller communicating with said enclosed specimen placement volume and being operative to maintain said enclosed specimen placement volume at a pressure which exceeds a vapor pressure of a liquid sample in said specimen placement volume and is greater than a pressure of a volume outside said enclosure, whereby a pressure differential across said cover does not exceed a threshold level at which rupture of said cover would occur.
- 15 2. A specimen enclosure assembly according to claim 1 and wherein said pressure controller comprises a passageway communicating with said enclosed specimen placement volume.
- 20 3. A specimen enclosure assembly according to claim 2 and wherein said passageway comprises a tube having a lumen whose cross section is sufficiently small as to maintain said pressure, which exceeds said vapor pressure of said liquid sample in said specimen placement volume and is greater than said pressure of said volume outside said enclosure, for a time period of at least 15 minutes.
- 25 4. A specimen enclosure assembly according to claim 3 and wherein said tube communicates with a fluid reservoir.
- 30 5. A specimen enclosure assembly according to any of the preceding claims 1-4 and also comprising a liquid ingress and egress assembly permitting supply and removal of liquid from said enclosed specimen placement volume.

6. A specimen enclosure assembly according to claim 5 and wherein said liquid ingress and egress assembly comprises at least two tubes.

7. A specimen enclosure assembly for use in an electron microscope and 5 comprising:

a rigid specimen enclosure dish having an aperture and defining an enclosed specimen placement volume;

an electron beam permeable, fluid impermeable, cover sealing said specimen placement volume at said aperture from a volume outside said enclosure; and

10 a liquid ingress and egress assembly permitting supply and removal of liquid from said enclosed specimen placement volume.

8. A specimen enclosure assembly according to claim 7 and wherein said liquid ingress and egress assembly comprises at least two tubes.

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9. A scanning electron microscope assembly comprising:

a scanning electron microscope defining an examination volume;

a specimen enclosure assembly disposed in said examination volume and including:

20 a rigid specimen enclosure dish having an aperture and defining an enclosed specimen placement volume;

an electron beam permeable, fluid impermeable, cover sealing said specimen placement volume at said aperture from a volume outside said enclosure; and

25 a pressure controller communicating with said enclosed specimen placement volume and being operative to maintain said enclosed specimen placement volume at a pressure which exceeds a vapor pressure of a liquid sample in said specimen placement volume and is greater than a pressure of a volume outside said enclosure, whereby a pressure differential across said cover does not exceed a threshold level at which rupture of said cover would occur.

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10. A scanning electron microscope assembly according to claim 9 and wherein said pressure controller comprises a passageway communicating with said

enclosed specimen placement volume.

11. A scanning electron microscope assembly according to claim 10 and wherein said passageway comprises a tube having a lumen whose cross section is sufficiently small as to maintain said pressure, which exceeds said vapor pressure of said liquid sample in said specimen placement volume and is greater than said pressure of said volume outside said enclosure, for a time period of at least 15 minutes.
12. A scanning electron microscope assembly according to claim 11 and wherein said tube communicates with a fluid reservoir.
13. A scanning electron microscope assembly according to any of the preceding claims 9-12 and also comprising a liquid ingress and egress assembly permitting supply and removal of liquid from said enclosed specimen placement volume.
14. A scanning electron microscope assembly according to claim 13 and wherein said liquid ingress and egress assembly comprises at least two tubes.
- 20 15. A specimen enclosure assembly for use in an electron microscope and comprising:
  - a fluid reservoir;
  - a plurality of rigid specimen enclosure dishes, each having an aperture and defining an enclosed specimen placement volume, said plurality of rigid specimen enclosure dishes communicating with said fluid reservoir;
  - 25 an electron beam permeable, fluid impermeable, cover sealing each said specimen placement volume at said aperture from a volume outside each said enclosure; and
  - a pressure controller communicating with said fluid reservoir and being operative to maintain said enclosed specimen placement volumes at a pressure which exceeds a vapor pressure of a liquid sample in said specimen placement volumes and is greater than a pressure of a volume outside said fluid reservoir, whereby a pressure

differential across said covers does not exceed a threshold level at which rupture of said covers would occur.

16. A specimen enclosure assembly according to claim 15 and wherein said  
5 pressure controller comprises a passageway.

17. A specimen enclosure assembly according to claim 16 and wherein said  
passageway comprises a tube having a lumen whose cross section is sufficiently small  
as to maintain said pressure, which exceeds said vapor pressure of said liquid sample in  
10 said specimen placement volume and is greater than said pressure of said volume  
outside said plurality of enclosures, for a time period of at least 15 minutes.

18. A specimen enclosure assembly according to claim 17 and wherein said  
tube communicates with said fluid reservoir.

15

19. A specimen enclosure assembly according to any of the preceding  
claims 15-18 and also comprising a liquid ingress and egress assembly permitting  
supply and removal of liquid from said enclosed specimen placement volume.

20 20. A specimen enclosure assembly according to claim 19 and wherein said  
liquid ingress and egress assembly comprises at least two tubes.

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FIG. 1

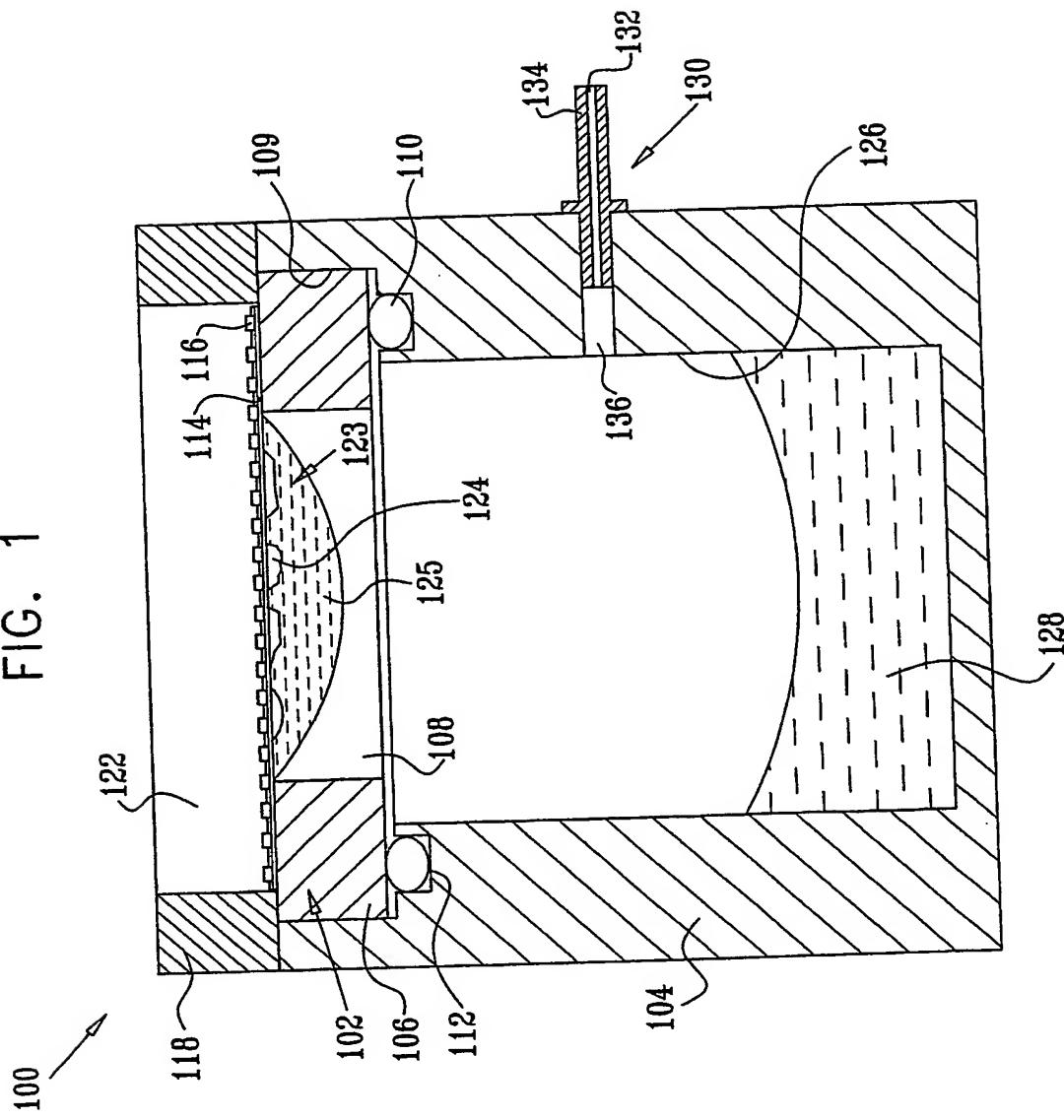
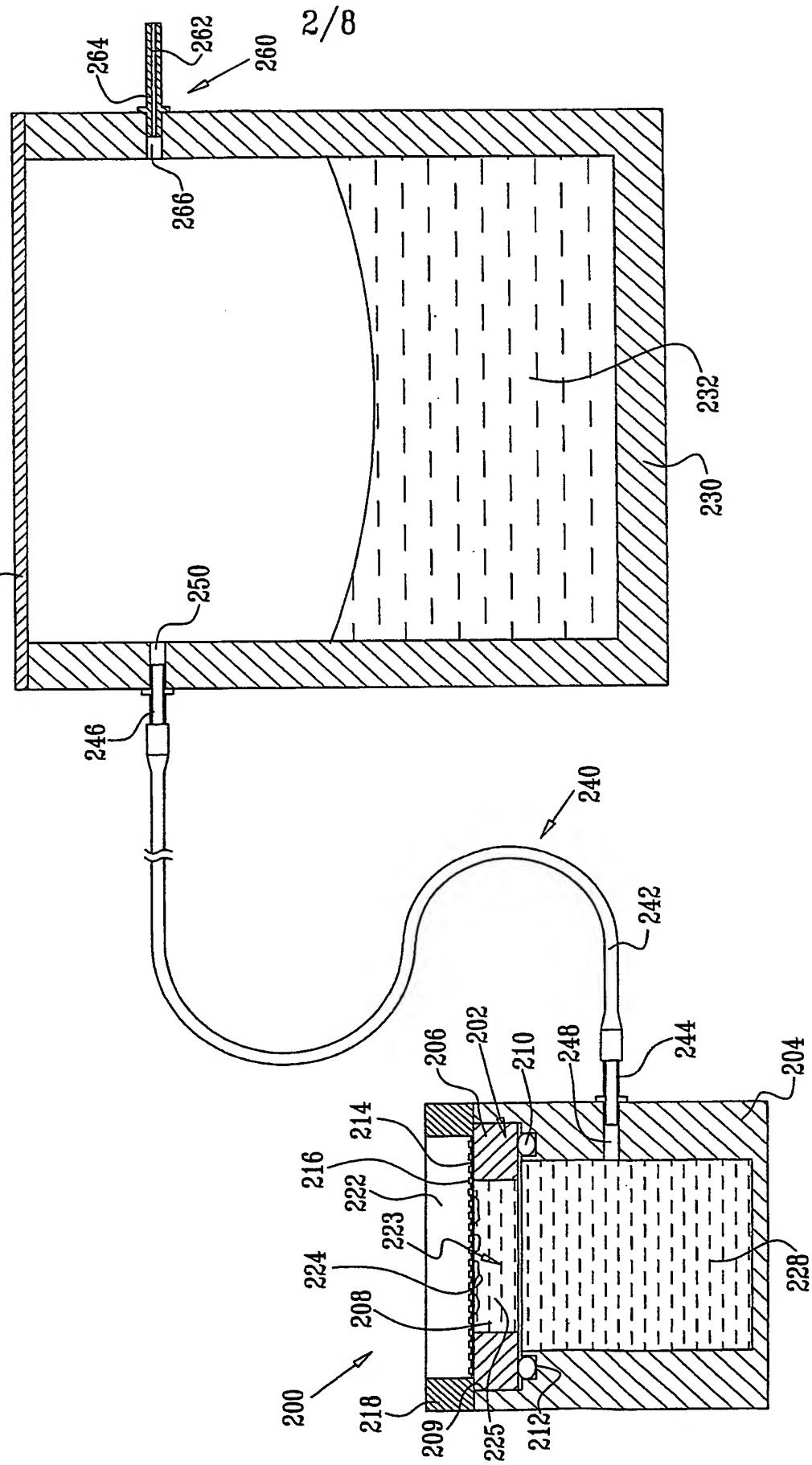
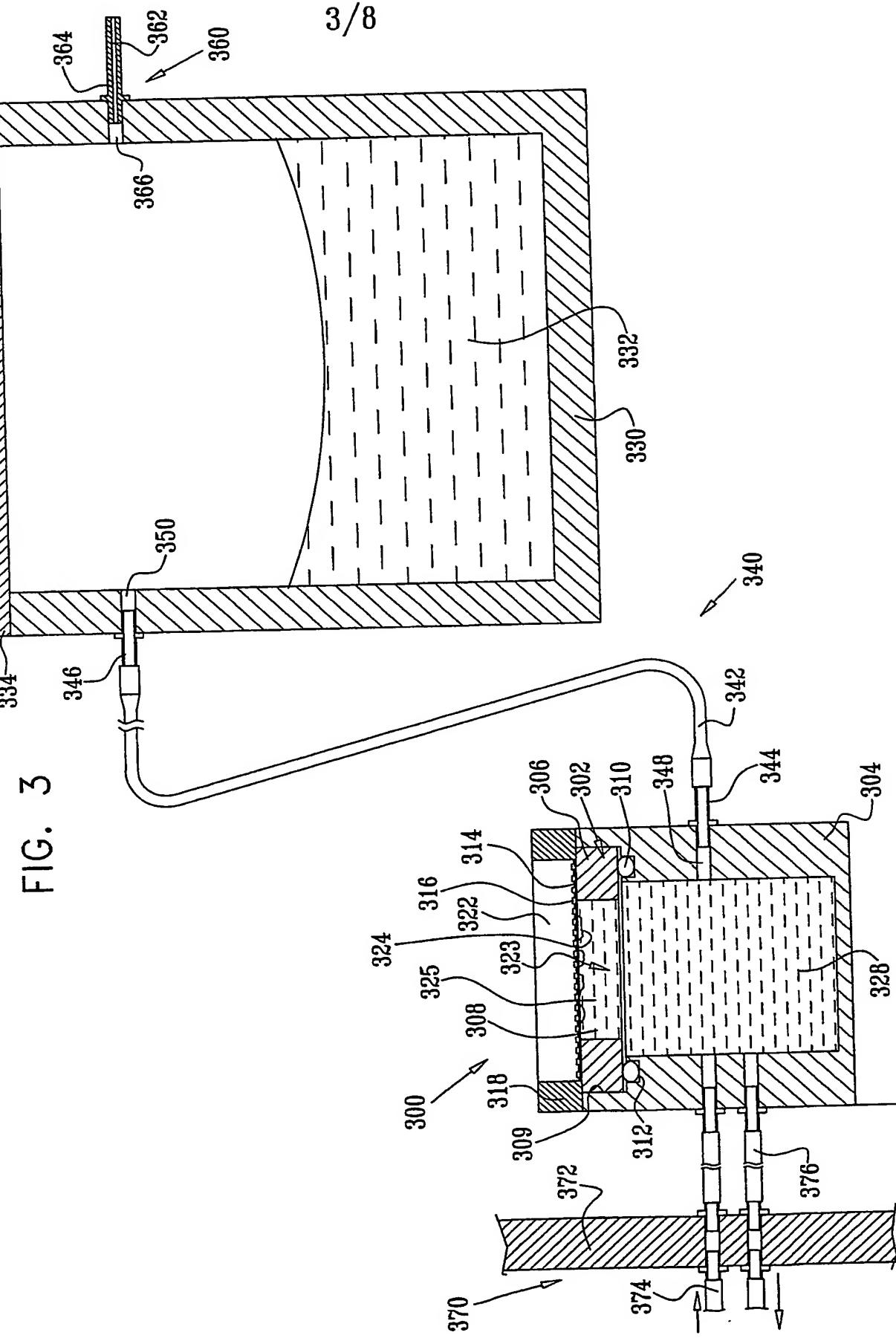
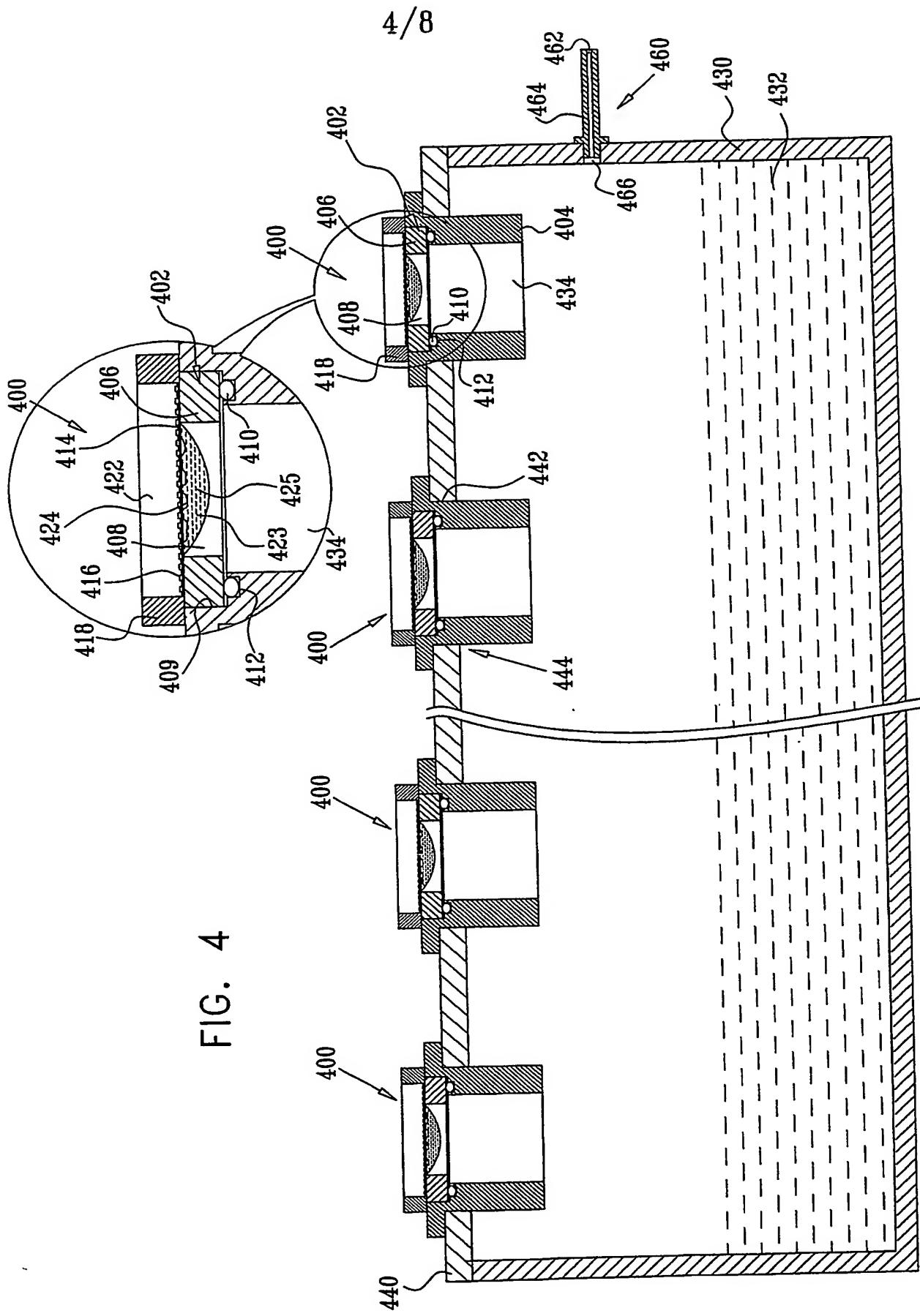


FIG. 2







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FIG. 5

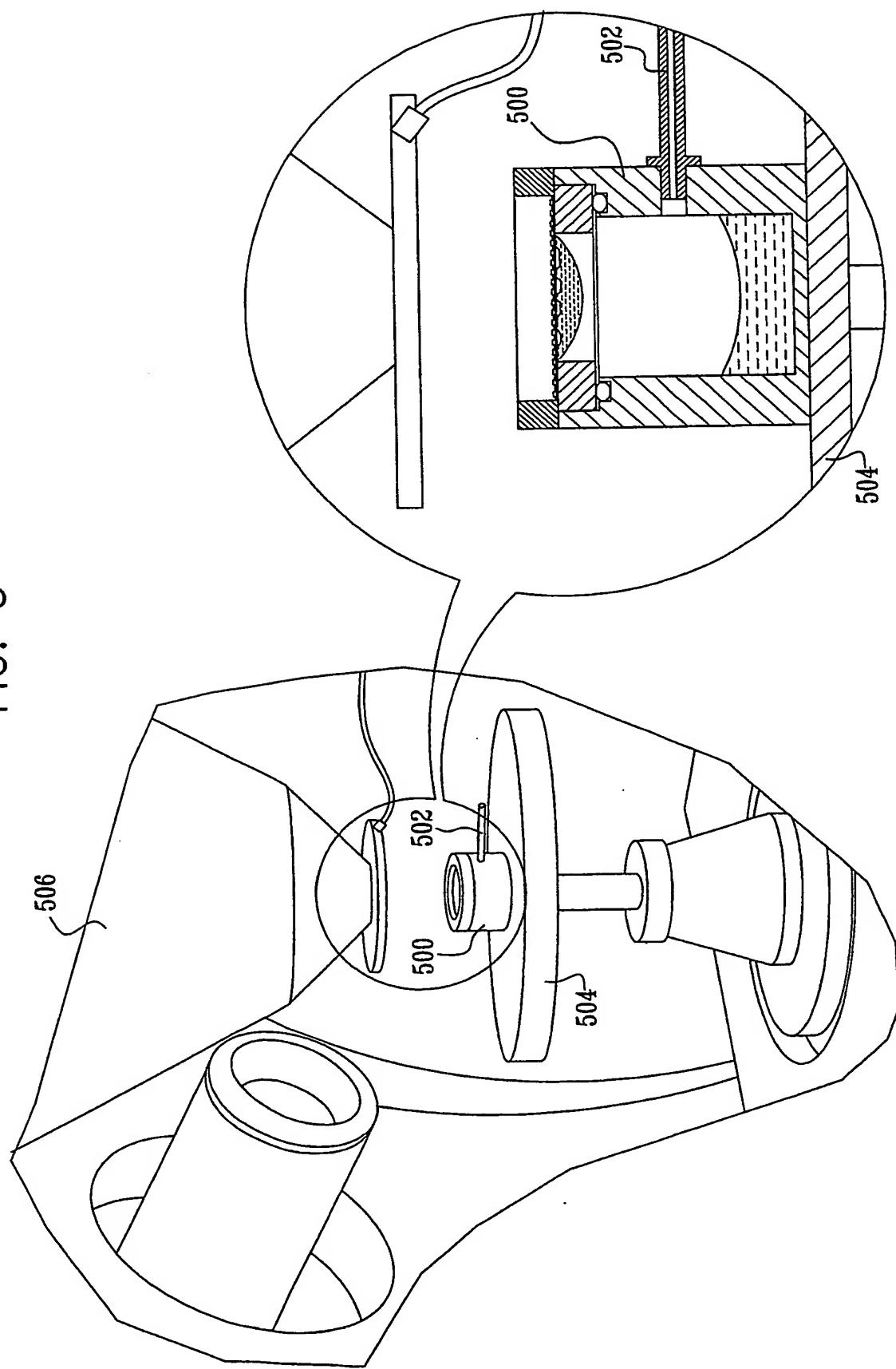


FIG. 6

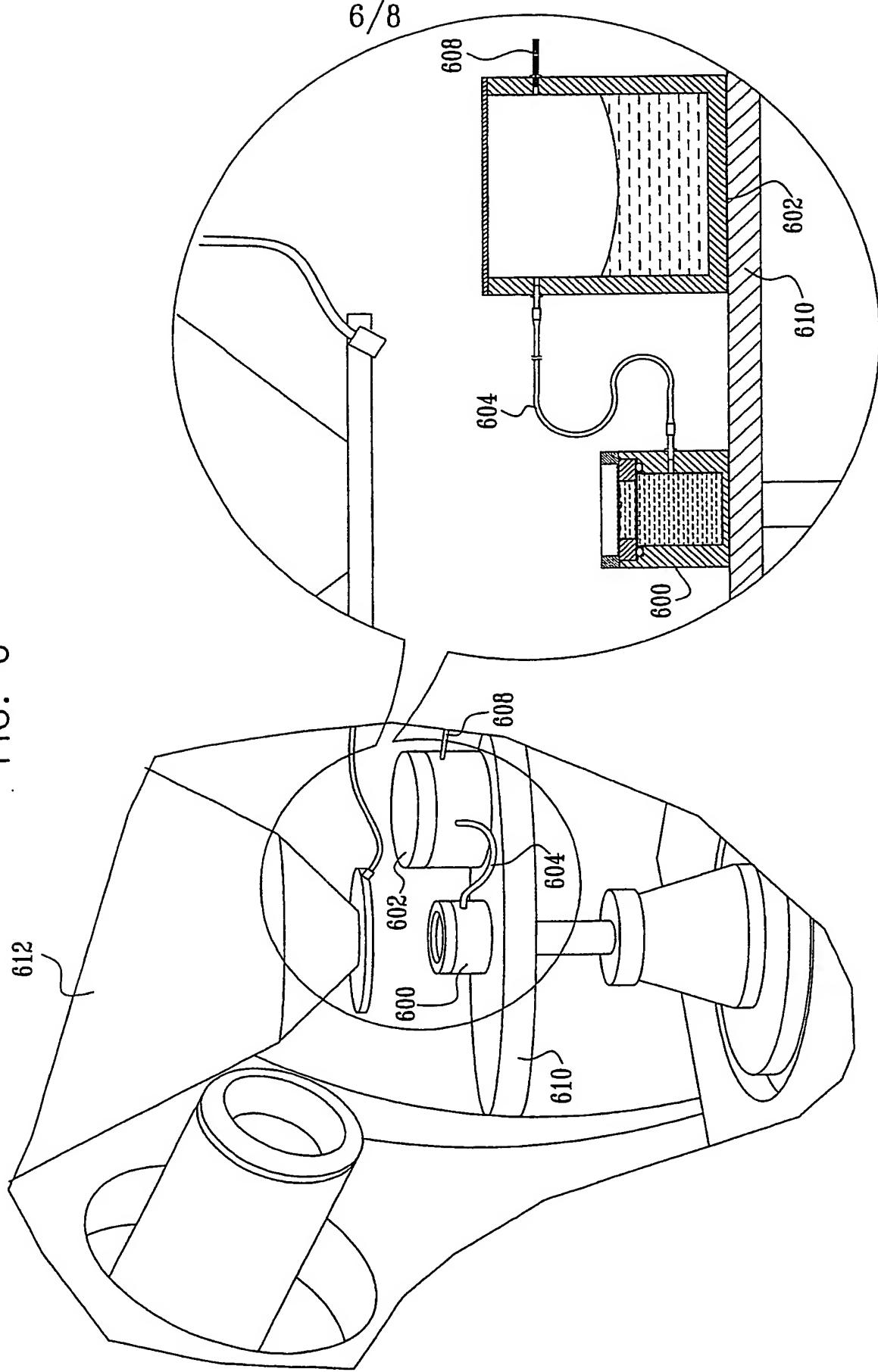


FIG. 7

